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2) **Over-** expression **of the** MDM2 **gene is found in some cases of haematological** malignancies .
Quesnel B; Preudhomme C; Oscier D; Lepelley P; Collyn-d'Hooghe M; Facon T ; Zandecki M; Fenaux P
Inserm U124, Institut de Recherches sur le Cancer de Lille, France.
British journal of haematology (ENGLAND) Oct **1994** , 88 (2) p415-8, ISSN 0007-1048 Journal Code: 0372544

3) **Amplification of the MDM2 gene in human breast** cancer **and its association with MDM2 and** p53 **protein status.**
McCann A H; Kirley A; Carney D N; Corbally N; Magee H M; Keating G; Dervan P A
Biotechnology Centre, University College Dublin, Belfield, Ireland.
British journal of cancer (SCOTLAND) May **1995** , 71 (5) p981-5, ISSN 0007-0920 Journal Code: 0370635

4) **Frequent occurrence of** p53 **mutations in rhabdomyosarcoma and leiomyosarcoma, but not in fibrosarcoma and** malignant **neural tumors.**
Wurl P; Taubert H; Bache M; Kroll J; Meye A; Berger D; Siermann A; Holzhausen H J; Hinze R; Schmidt H; Rath F W
Surgical Clinic, Martin Luther University of Halle-Wittenberg, Halle/S., Germany.
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FREQUENT OCCURRENCE OF *p53* MUTATIONS IN RHABDOMYOSARCOMA AND LEIOMYOSARCOMA, BUT NOT IN FIBROSARCOMA AND MALIGNANT NEURAL TUMORS

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We have analyzed soft-tissue sarcomas (STS) molecularly for mutations in the tumor-suppressor gene *p53* and immunohistochemically for expression of p53 and mdm2 proteins. In this study, tumor samples from 3 groups of soft-tissue sarcomas, *i.e.*, fibrosarcomas, myogenic sarcomas and malignant neural tumors (MNT), were investigated. The methods applied encompass immunohistochemistry on 198 tumor samples using p53 antibodies (DO-1 and DO-7) and an mdm2 antibody (IF-2). Out of these, 100 samples were subjected to non-radioactive PCR-SSCP-sequencing analysis. Immunohistochemical detection rate for p53 (range of 57% to 67%) and for mdm2 proteins (range of 19 to 44%) was similar in all 3 groups. In higher tumor grades, an increased rate of immunopositivity was found for p53 but not for mdm2. Investigation of *p53* mutational status revealed 6 mutations in myogenic sarcomas but none in malignant neural tumors or fibrosarcomas, suggesting different roles of *p53* in the 3 STS groups. Interestingly, a G → A transition in codon 245 (a CpG site) was found in 3 myogenic sarcomas. Our results and those of others suggest *p53* codon 245 as a mutational hotspot in sarcomas, as recognized in carcinomas.
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Soft-tissue sarcomas (STS) can be defined as malignant tumors of non-epithelial extraskeletal tissue, excluding the reticulo-endothelial system, glia, and supporting tissue of various parenchymal organs. By convention, malignant tumors of the peripheral nervous system are included (Enzinger *et al.*, 1969). In addition to MFH's and liposarcomas, the most frequent are fibrosarcomas (8–12%), malignant peripheral-nerve-sheath tumors (8–10%), peripheric neuroblastomas (5%), leiomyosarcomas (5–10%), and rhabdomyosarcomas (10–20%) (Enzinger *et al.*, 1969; Enjoji and Hashimoto, 1984). However, even after distinct tumor classification, there are no comprehensive immunohistochemical and molecular data that convincingly characterize malignant tumors according to the course of disease and the prognosis. Apart from oncogenes, tumor-suppressor genes, in particular, and their role in cell-cycle regulation are of crucial interest. Among tumor suppressors, *p53* stands out, with about 50% of mutational alterations in malignomas (Hollstein *et al.*, 1991, 1996). *p53* mutational analysis for soft-tissue sarcomas has been performed for fibrosarcomas (Latres *et al.*, 1994), neuroblastomas (Imamura *et al.*, 1993; Komuro *et al.*, 1993; Vogan *et al.*, 1993; Hosoi *et al.*, 1994), neurofibrosarcomas (Menon *et al.*, 1990), leiomyosarcomas (Stratton *et al.*, 1990; Andreassen *et al.*, 1993; Liu *et al.*, 1994; Latres *et al.*, 1994; Patterson *et al.*, 1994; Cordon-Cardo *et al.*, 1994; De Vos *et al.*, 1994), rhabdomyosarcomas (Stratton *et al.*, 1990; Cordon-Cardo *et al.*, 1994; Mulligan *et al.*, 1990; Castresana *et al.*, 1995; Felix *et al.*, 1992), MFH's and liposarcomas (reviewed in Taubert *et al.*, 1995), and other STS (Liu *et al.*, 1994; Cordon-Cardo *et al.*, 1994; Toguchida *et al.*, 1992; Boman *et al.*, 1994; Scinicariello *et al.*, 1994; Dumaz *et al.*, 1993; Hollstein *et al.*, 1994). In carcinomas, the great majority of *p53* mutations are missense mutations, and out of these about 40% occur at mutational hot spots (Levine, 1993). On closer examination, one third of 280 tumor mutations was found to consist of transitions at hot-spot regions with CpG sites (Hollstein *et al.*, 1991). On investigating the mutational spectrum for soft-tissue sarcomas, we found a spectrum similar to that of carcinomas (Taubert *et al.*, 1995). Most of the mutations are missense mutations, with the majority occurring at CpG sites.

In addition to mutations in the *p53* gene, an amplification of the *mdm2* oncogene affects sarcoma tumorigenesis. Amplification of the *mdm2* gene results in mdm2 protein over-expression with complexing and inactivating p53 protein (Momand *et al.*, 1992). It was detected in liposarcomas, MFH's (Oliner *et al.*, 1992; Leach *et al.*, 1993), leiomyosarcomas (Patterson *et al.*, 1994), a group of different soft-tissue sarcomas (Cordon-Cardo *et al.*, 1994) and osteosarcomas (Oliner *et al.*, 1992; Ladanyi *et al.*, 1993). Immunohistochemical detection of mdm2 over-expression revealed that in most cases positive staining is alternative to *p53* alterations over-expression (Leach *et al.*, 1993), but we found co-existing over-expression, earlier described for soft-tissue sarcomas (Cordon-Cardo *et al.*, 1994).

The goal of our study was to extend the mutational analysis for *p53* on 3 sarcoma entities with high occurrence: myogenic sarcoma (Leiomyosarcoma and rhabdomyosarcoma), fibrosarcoma and malignant neural tumors. We also examined these tumors for alterations in K-*ras* and N-*ras* genes, in order to more fully comprehend the mutational spectrum in malignant soft-tissue tumors. Additionally, we tested different monoclonal antibodies (MAbs) against p53 and mdm2 for a large number of patients and tumor specimens. The immunohistochemical and mutational data were considered in relation to clinical data, to improve understanding of their clinical relevance.

MATERIAL AND METHODS

Tumor samples

A collection of 198 tumor samples originating from formalin-fixed paraffin-embedded STS from 127 patients (Institute of Pathology and Surgical Clinic, University of Halle, Germany) were chosen for immunohistochemistry (IHC). Of these, 100 samples from 78 patients were investigated for *p53* mutations by PCR-SSCP-sequencing analysis (Table I). For K-*ras* mutation, we investigated 32 samples from myogenic sarcomas (21 patients) and, for detection of N-*ras* mutation, 31 MNT samples (25 patients) were examined. All the patients had had local radical surgical treatment. From the patients involved, clinical data, including the survival rate, were collected and grading was performed, taking into consideration mitotic activity and verification of tumor necrosis (Van Unnik *et al.*, 1988). Patients average post-treatment observation periods were 47 months (7 to 130) for fibrosarcomas, 22 months (2 to 107) for malignant neural tumors, 30 months (1 to 156) for leiomyosarcomas and 17 months (7 to 30) for rhabdomyosarcomas.

Immunohistochemistry

Immunohistochemical staining for p53 was done for all tumor samples, using MAbs DO-1 (Oncogene Science, Manhasset, NY) and DO-7 (Medac, Hamburg, Germany). Samples

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with *p53* mutations were characterized additionally by a polyclonal antibody CM-1 (Medac) and 2 other MAbs Pab 1801, Pab 240 (Oncogene Science) as described (Taubert *et al.*, 1995). For mdm2 we used the MAb IF2 (Oncogene Science) recognizing an N-terminal epitope. IF-2 was used at a working solution of 5 μg/ml. All other steps of staining were the same as described (Taubert *et al.*, 1995). As positive control for mdm2 the osteosarcoma cell line Saos-2 and for p53 Pank TuI were used. As negative control, the first antibody was omitted and replaced by an unrelated MAb of the same isotype in the same concentration. Staining for all antibodies was considered positive if more than 10% of the cells showed distinct reactivity. If more than one antibody was used for the same antigene, positive staining of one antibody is sufficient for positivity (for *p53* only, Do-1 or/and Do-7).

DNA isolation and PCR

The DNA from paraffin sections was isolated according to standard methods and PCR for exons 4 to 9 of the *p53* gene was performed as described (Taubert *et al.*, 1995). PCR for exons 1 and 2 of the K-*ras* gene (Grimmond *et al.*, 1992) and the N-*ras* gene (Syvänen *et al.*, 1992) was performed as reported.

SSCP analysis and DNA sequencing

Non-radioactive SSCP analysis and DNA sequencing are described in detail (Thamm, 1995). Briefly: for SSCP analysis, 10 μl (approx. 1.5 μg) of PCR product were dissolved in SSCP buffer (98% formamide, 20 mM EDTA, 0.05% bromophenol blue), denatured for 5 min at 98°C and immediately stored on ice. The samples were run in 6% or 10% non-denaturing ready-made TBE-gels (Novex, San Diego, CA) at 9 to 13°C for 2.5 to 3 hr (85–100 V). Afterwards, the gels were silver-stained according to standard protocols (silver-staining kit, Promega, Madison, WI) to detect shifts in the single-strand-DNA pattern. For sequencing, the purified PCR products were amplified by a cyclic PCR using the corresponding 5'-biotinylated primers, and sequencing products were verified by chemiluminescence (CPD-Star, Tropix, Bedford, MA).

Allele-specific oligonucleotide hybridization (ASO)

The PCR products from genomic DNA of the patients and the control were denatured by heat and immediately stored on ice. Samples and the control (5 μl each) were spotted on a nylon membrane, dried and UV-cross-linked for 5 min. After pre-hybridization (1 hr, 65°C) in 50 ml hybridization solution (10 × Denhardt's, 2 × SSC, 0.1% SDS), 100 ng 3'-biotin-labelled probe were added to 50 ml of freshly prepared hybridization solution (heat-denatured) and incubated overnight at 65°C. Washing of the membrane (15 min, 2 × SSC, 2 × 15 min, 1 × SSC) was performed at special temperatures (64°C, 68°C and 72°C). For the detection of DNA, a hybridization chemiluminescence assay (CPD-Star, Tropix) was applied.

Wild-type probe: E7wil24

5'-TCCGGTTCATGCCGCCCATGCAGGG-3'

Mutant probe: E7mut24

5'-TCCGGTTCATGCTGCCCATGCAGGG-3'

TABLE I – CHARACTERISTICS OF THE SOFT-TISSUE TUMOR SAMPLES[1]

Soft-tissue tumors	Myogenic sarcoma	Malignant neural tumors	Fibrosarcoma	Total
Tumor samples	54[2] (35)[3]	63[4] (31)[5]	81 (34)	198 (100)[6]
Primary tumors	30 (17)	38 (16)	40 (17)	108 (50)
Recurrences	15 (9)	17 (10)	38 (15)	70 (34)
Metastases	9 (9)	8 (5)	3 (2)	20 (16)
Grade 1	2 (2)	1 (1)	13 (6)	16 (9)
Grade 2	24 (19)	30 (14)	29 (18)	83 (51)
Grade 3	28 (14)	32 (16)	39 (10)	99 (40)
Patients	37 (26)	42 (25)	48 (27)	127 (78)
Patients alive	10 (6)	8 (3)	20 (8)	38 (17)
Patients dead	27 (20)	34 (22)	28 (19)	89 (61)

[1]Tumor samples and number of patients with soft-tissue tumors investigated immunohistochemically and (in parentheses) molecularly.–[2]Includes 12 rhabdomyosarcoma (9 patients) and 42 leiomyosarcoma samples (28 patients). All but one of the rhabdomyosarcomas were adult, pleomorphic tumors.–[3]Includes 6 rhabdomyosarcoma (3 patients) and 29 leiomyosarcoma samples (23 patients).–[4]Consisting of 52 neurogenic sarcoma (33 patients) and 11 peripheric neuroblastoma samples (9 patients).–[5]Consisting of 22 neurogenic sarcoma (20 patients) and 9 peripheric neuroblastoma samples (5 patients).–[6]In most cases, a tumor is represented by one sample; a maximum of 3 samples originated from one tumor.

RESULTS

Immunohistochemistry

Three groups of malignant STS — fibrosarcomas, malignant neural tumors (peripheric neuroblastoma and malignant PNST) and myogenic sarcomas (rhabdo- and leiomyosarcomas) — were investigated for *p53* and mdm2 protein, and their relationship to the grading was recorded (Table II). A total of 198 samples from 127 patients originated from 54 myogenic sarcomas (12 rhabdomyosarcomas and 42 leiomyosarcomas from 9 and 28 patients respectively), 63 malignant neural tumors (42 patients) and 81 fibrosarcomas (48 patients).

p53 *immunoreactivity*

Of the tumor samples, 57% (113/198) showed immunohistochemically p53-positive after staining with DO-1 and/or DO-7 MAbs (Fig. 1 and 4). Separating the samples according to tumor entities, 75% of the rhabdomyosarcomas (9/12), 57% of the leiomyosarcomas (24/42), 54% of the malignant neural tumors (34/63) and 57% of the fibrosarcomas (46/81) showed p53 positivity (Table II). Among these, 26% (4/15) grade-I, 58% (46/80) grade-II and 61% (63/103) grade-III samples were found; this reveals a correlation between increasing malignancy and the number of *p53*-positive tumors.

TABLE II – RESULTS OF IMMUNOHISTOCHEMICAL ANALYSIS FOR TUMOR SAMPLES OF THE TUMOR GROUPS MYOGENIC SARCOMAS, MALIGNANT NEURAL TUMORS AND FIBROSARCOMAS

STS samples positive/total	Myogenic sarcoma Rhab	Myogenic sarcoma Leio	Malignant neural tumors	Fibrosarcoma	Total
p53[1]	9/12	24/42	34/63	46/81	113/198
Grade 1	0/0	0/1	0/1	4/13	4/15
Grade 2	1/2	10/22	21/30	14/26	46/80
Grade 3	8/10	14/19	13/32	28/42	63/103
mdm2 (IF-2)	4/11	6/43	15/63	36/81	61/198
Grade 1	0/0	0/1	0/1	8/13	8/15
Grade 2	0/2	5/22	9/30	10/27	24/81
Grade 3	4/9	1/20	6/32	18/41	29/102

[1]Staining of p53 antibodies Do 1 and/or Do 7 was considered as positive.–Rhab, rhabdomyosarcoma; Leio, leiomyosarcoma.

mdm2 immunoreactivity

IF-2 was positive in 31% (61/198) of the tumor samples immunohistochemically studied. Positive staining was observed in 36% (4/11) of the rhabdomyosarcomas, 14% (6/43) of the leiomyosarcomas, 24% (15/63) of the MNT and 44% (36/81) of the fibrosarcomas (Table II, Fig. 1 and 6).

In contrast to the findings of Cordon-Cardo *et al.* (1994), mdm2 over-expression could not be related to a higher tumor grade, as shown also by Wiethege *et al.* (1994). However, over-expression of mdm2 protein occurs in all investigated soft-tissue entities, confirming the role of mdm2 over-expression in soft-tissue tumorigenesis (Leach *et al.*, 1993).

Mutational analysis for p53 *(exons 4 to 9)*

On investigation, 3 groups of soft-tissue sarcomas for *p53* mutations (exons 4 to 9), no such mutations could be identified for the MNT and fibrosarcoma entities (31 and 34 samples respectively), but mutations were detected in the group of myogenic sarcomas, *i.e.*, in leiomyosarcoma as well as in rhabdomyosarcoma samples.

For 10/35 myogenic-sarcoma samples (5 from primary tumors, 4 from recurrences and one metastasis) from 6/26 patients, 4 different mutations were recorded. These mutations were G-to-A transitions in codon 158 and 245 respectively, a 1-bp insertion in codon 215 and a 15-bp deletion in exon 5 (Table III, Fig. 2). Surprisingly, 6 myogenic sarcoma samples from 3 patients carried the same mutation, a G-to-A transition in codon 245 (exon 7) (Table III). The samples originated from one recurrence (M28), 3 biopsies of a recurrence (M19, M20, M21) and 2 biopsies of a primary tumor (M24, M25) respectively. Samples M20 and M28 showed unambiguously the transition after sequencing. However, samples M19 and M21 (from the same tumor as M20) were not unambiguous, showing a very weak sequencing signal for the transitional base exchange. Consequently, allele-specific oligonucleotide hybridization (ASO) was applied, using mutant-specific (E7mut24) and wild-type-specific probes (E7wil24).

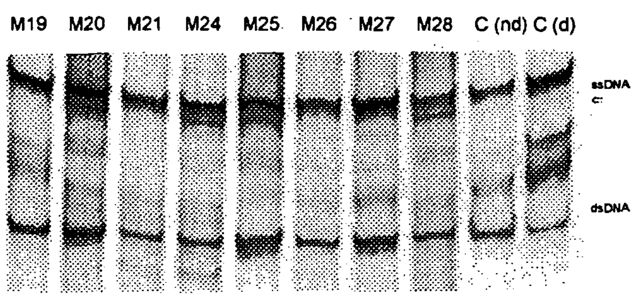

FIGURE 1 – Result of SSCP analyses of exon 7 from the *p53* gene for myogenic sarcomas. A shift in the ssDNA pattern was observed in samples M20, M24, M25 and M28 (marked by an arrowhead). [a]c-control, ssDNA, single-strand DNA; dsDNA, double-strand DNA. [b]nd-not denatured, d-denatured.

The mutant-specific probe hybridized at 68°C only with potentially mutated DNA samples (M19/M20/M21, M28), but not with normal control DNA (Fig. 3). The wild-type-specific probe, on the other hand, hybridized with all DNA samples, because of wt-*p53*-alleles or remnants of normal cells (*e.g.*, infiltrating lymphocytes and vessels) still present in the sample (data not shown). The G-to-A transition was confirmed by repeating the ASO experiments, the PCR and sequencing reactions independently at least twice.

For one primary tumor of a leiomyosarcoma (M44) and its metastasis (M45) a G-to-A transition in codon 158 (exon 5)

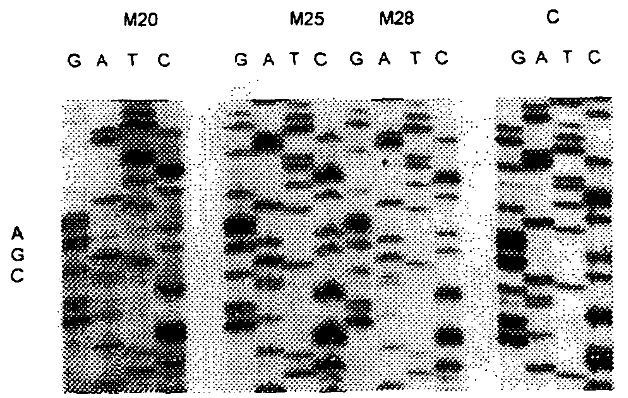

FIGURE 2 – Results of sequencing of myogenic sarcomas with a point mutation in codon 245. In all 3 tumor samples a GGC to AGC transition was identified in nucleotide 733.

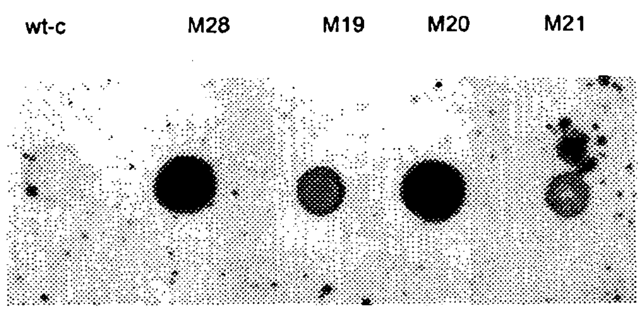

FIGURE 3 – Results of the dot-blot of tumor samples from myogenic sarcomas (M28, M19, M20, M21) with wild-type-specific (wt-KE7) and mutant-type-specific oligonucleotides (mt-KE7) of PCR products for exon 7 of the *p53* gene. Hybridization with mutant-specific oligonucleotides (see "Material and Methods") at 68°C shows a specific signal for the tumor samples M28 (transition in codon 245 identified by sequencing) and M19, M20, M21. The control DNA (wt-cj from peripheral-blood cells) shows only a very weak signal.

TABLE III – RESULTS OF MOLECULAR ANALYSIS FOR MYOGENIC SARCOMAS WITH A *p53* MUTATION

Tumor sample	Entity	Grade	P/R/M	sv	ex	nt	codon	alt	bp-alt	aa-alt
M42	Leio	III	P	d	4	318–332	106–111	del	–(15)	5-aa-del
M44/M45	Leio	II/II	P/M	d	5	473	158	ts	CGC → CAC	Arg → His
P6-93	Leio	III	P	a	6	643	215	ins	+ (1)	frameshift[1]
M24/M25	Leio	II/II	P/P	d	7	733	245	ts	GGC → AGC	Gly → Ser
M28	Rhab	II	R	d	7	733	245	ts	GGC → AGC	Gly → Ser
M19/M20/M21	Rhab	III/III/II	R/R/R	d	7	733	245	ts	GGC → AGC	Gly → Ser

All identified *p53* mutations are in the region of codons 106–245, and therefore concern the core protein domain (codons 100–300). The high portion of transitional-point mutations is striking. This was also observed for other soft-tissue tumor entities.

[1]The insertion identified results in a frameshift and a new stop codon (codon 221).–P, primary tumor; R, recurrence; M, metastases; sv, survival; ex, exon; nt, nucleotide; bp, base pair(s); aa, amino acid(s); alt, alteration; del, deletion; a, alive; d, dead; Rhab, rhabdomyosarcoma; Leio, leiomyosarcoma; ts, transition; ins, insertion.

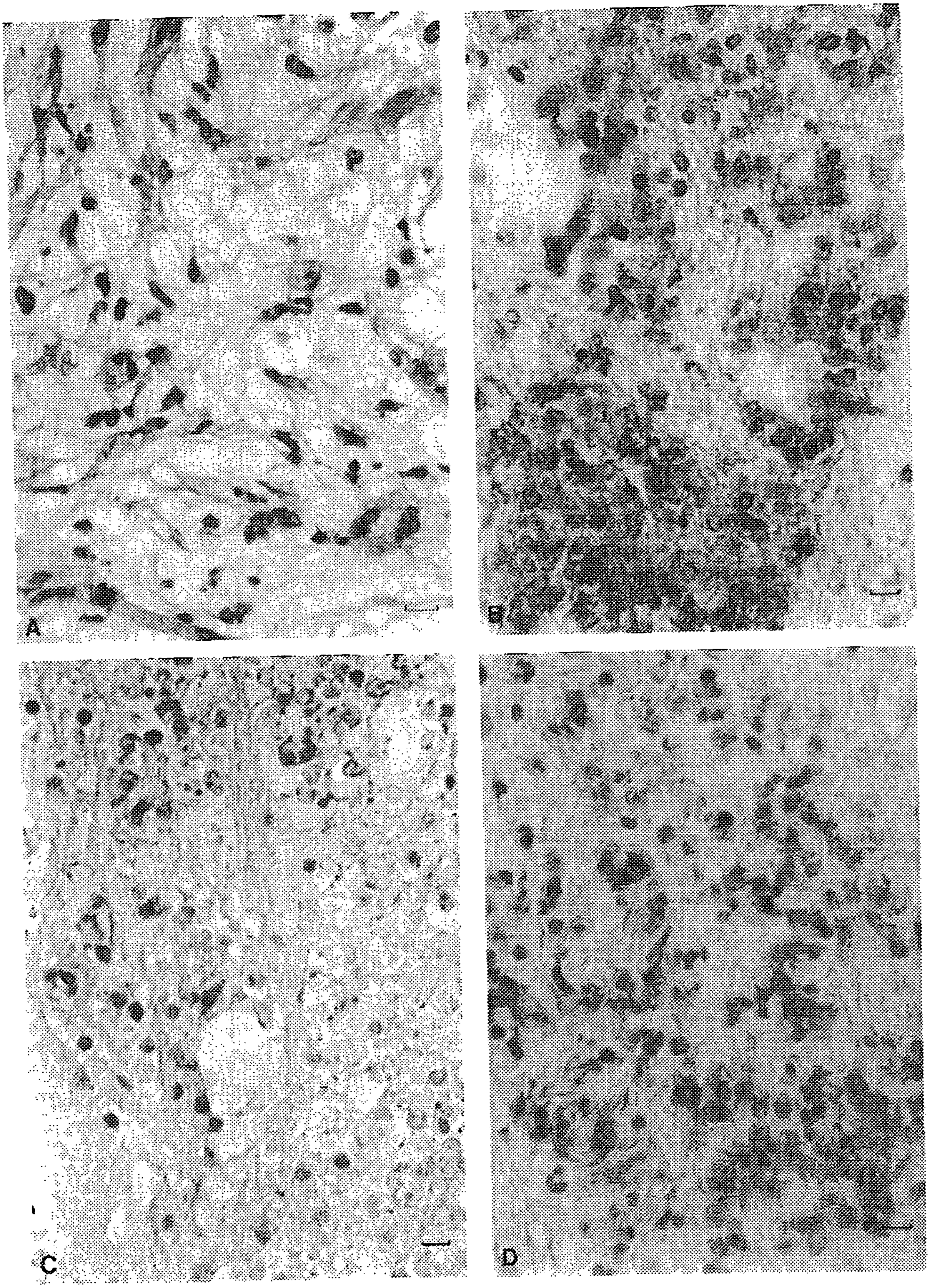

FIGURE 4

TABLE IV - RESULTS OF IMMUNOHISTOCHEMISTRY FOR p53 AND mdm2 IN MYOGENIC SARCOMA SAMPLES WITH p53 MUTATIONS

Sample	Grade	p53					mdm2
		CM-1	DO-1	DO-7	Pab1801	Pab240	IF-2
M 19	III	+	−	−	−	+	−
M 20	III	+	++	++	+	++	++
M 21	II	++	−	−	−	++	−
M 24	II	++	++	++	++	++	−
M 25	II	++	++	++	++	+	−
M 28	II	++	++	++	++	+	
M 42	III	+	+	−	−	+	−
M 44	II	−	++	++	+	+	−
M 45	II	+	++	++	+	−	+
P6-93	III	++	++	++	−	++	++

Assessment of applied antibodies: −, no expression; +, distinct expression; ++, strong expression.

was identified, pointing to a selective advantage of clones with this *p53* mutation. Furthermore, a primary tumor of a leiomyosarcoma (P6-93) carried a 1-bp insertion in codon 215, causing a frame shift resulting in a stop in codon 221. This alteration was also detectable in a simultaneously established primary-cell culture of the same tumor (data not shown). Additionally, in one leiomyosarcoma sample (M42) a 15-bp deletion (codons 106–111) was detected, resulting in the loss of 5 amino acids and an amino-acid exchange from Ser to Arg in codon 106. In all sequencing reactions, the mutated sequence as well as the wt-sequence could be found as described above.

In a PCR-SSCP-sequencing analysis of exons 1 and 2 from the K-*ras* gene in myogenic sarcomas (32 tumor samples from 21 patients) and of exons 1 and 2 from the N-*ras* gene in MNT (31 tumor samples from 25 patients), no mutation could be detected (data not shown).

DISCUSSION

Three groups of STS, *i.e.*, fibrosarcomas, malignant neural tumors and myogenic sarcomas, were analyzed molecularly and immunohistochemically.

Immunohistochemistry

The finding of 26% grade-I, 57% grade-II and 61% grade-III tumor samples with p53 positivity is comparable to other STS, such as MFH and liposarcoma, where grade-II and grade-III tumors in particular showed p53 positivity (Kawai *et al.*, 1994; Taubert *et al.*, 1995).

The result of 32% mdm2 positivity is similar to the findings of Cordon-Cardo *et al.* (1994), who found positive staining in 37% (76/207) of STS. Unfortunately, investigation for *mdm2*-gene amplification was not possible, since paraffin-embedded material was studied. However, the finding that all of the 33% (8/24) STS with *mdm2*-gene amplification also showed mdm2 over-expression (Leach *et al.*, 1993) suggests gene amplification as a possible reason for mdm2 over-expression.

We were able to support that mdm2 expression is more abundant in metastases than in primary tumors (Ladanyi *et al.*, 1993; Cordon-Cardo *et al.*, 1994), since mdm2 positivity was detected in M45 (metastasis), but not in M44 (primary tumor of the same leiomyosarcoma).

Generally, a combination of p53 and mdm2 over-expression is recorded for all STS entities in this study. Over-expression of mdm2 has been discussed mainly as an alternative to p53 alteration/over-expression of mt-p53 through inactivating *p53* function (Leach *et al.*, 1993; Patterson *et al.*, 1994). However, cases of simultaneous over-expression for p53 and mdm2 have also been recorded (Cordon-Cardo *et al.*, 1994; Marston *et al.*, 1994). It is suggested that p53 protein over-expression may induce increased mdm2 RNA transcription (Florenes *et al.*, 1994), which could result in over-expression of mdm2 protein. Moreover, *p53*-mdm2 complexes could activate a function promoting tumorigenesis (Landers *et al.*, 1994).

Expression of mdm2 in *p53*-mutated myogenic sarcomas shows a heterogeneous picture. A 15-bp deletion (M42) showed no mdm2 positivity. An 1-bp insertional mutation was positive for mdm2, and the tumor samples with transitional mutations were in part mdm2-positive (Table IV). However, the latter result depended on the amount of tumor material (comparable to results in the sequencing reactions): for example, the M20 sample expressed strong mdm2 positivity, whereas M19 showed none. But at least one tumor sample (M20, M21, M25, M28, M45) from a patient with G-to-A transitions always showed mdm2 expression also. The co-existence of *p53* mutations and mdm2 positivity could be explained by a selective advantage in tumors of weaker phenotype (Ladanyi *et al.*, 1993).

Mutational analysis

Molecular characterization comprised a PCR-SSCP-sequencing analysis for the tumor-suppressor gene *p53* (exons 4 to 9) and in part for the N-*ras* and K-*ras* genes (exons 1 and 2).

Neither N-*ras* nor K-*ras* mutations could be detected. This agrees with other studies, which found no K-*ras* mutations (Pulciani *et al.*, 1982; Wilke and Robinson, 1993) and just 3 N-*ras* mutations in STS. All these mutations concerned codon 61 of exon 2, identified in a neuroblastoma, a fibrosarcoma and a rhabdomyosarcoma (Taparowsky *et al.*, 1983; Brown *et al.*, 1984; Chardin *et al.*, 1985). However, N-*ras*- and K-*ras*-gene mutations do not seem to play an important role in STS tumorigenesis.

p53 mutational analysis revealed 6 mutations in 35 myogenic sarcoma samples, but none in 34 fibrosarcoma and 31 MNT samples. In previous mutational studies, *p53* mutations appeared somewhat rarely in fibrosarcoma and MNT. The only *p53* mutation described in a fibrosarcoma (Latres *et al.*, 1994) represents an exceptional occurrence of *p53* mutations in this STS entity. In MNT, a small percentage may carry *p53* mutations, as shown for 4 cases of neuroblastomas (Imamura *et al.*, 1993; Vogan *et al.*, 1993; Hosoi *et al.*, 1994) and 2 cases of neurofibrosarcomas (Menon *et al.*, 1990). But mutational frequencies in the range of 11 to 18% verified for other soft-tissue entities (Stratton *et al.*, 1990, 10/94; Toguchida *et al.*, 1992, 3/17; Leach *et al.*, 1993, 4/24; Taubert *et al.*, 1995), were not recorded for fibrosarcomas and MNT.

For 10/35 tumor samples (6/29 leiomyosarcomas and 4/6 rhabdomyosarcoma samples) 6 mutations were identified. This

FIGURE 4 – Immunohistochemical staining of rhabdomyosarcoma M20 with anti-*p53* and anti-*mdm2* antibodies. *(a)* hemalaun/eosin staining; *(b)* anti-*mdm2* antibody IF-2; *(c)* anti-*p53* antibody DO-1; *(d)* anti-*p53* antibody DO-7. Scale bars, 10 µm.

result is comparable to the finding of 7 mutations in 26 myogenic sarcoma samples (4 mutations in 20 leiomyosarcomas and 2 mutations in 6 rhabdomyosarcomas) by Stratton *et al.* (1990). Unfortunately, no results concerning the patients are presented in this study. However, we found that 23% (6/26) of the myogenic sarcoma patients (4/23 of leiomyosarcoma patients and 2/3 of rhabdomyosarcoma patients) carried *p53* mutations.

In exon 4 we identified a 15-bp deletion (M42), in exon 5 a CGC-to-CAC transition for codon 158 (M44/M45), in exon 6 a 1-bp insertion in codon 215 and, surprisingly, in exon 7 a GGC-to-AGC transition in codon 245, which was identical in 3 patients (M19-21; M24/M25; M28). All mutations are located inside the core domain (codons 102–292; Cho *et al.*, 1994) and seem to affect structural rather than functional properties of the p53-DNA interaction.

All identified point mutations are G-to-A transitions and occur at CpG dinucleotides. It is known that CpGs are preferential loci for mutational hot spots. Although CpG sequences are under-represented in the human genome by the factor of 5, about 35% of point mutations causing human disorders occur at CpGs, and over 90% of these are transitions from G-to-A (Cooper and Youssoufian, 1988). In addition to causes such as differences in the fidelity and strand specificity of eucaryotic polymerases (Kunkel and Alexander, 1985; Wu and Maeda, 1987), cytosine methylation at CpG sites may cause the high mutational frequency. 5-methylatecytosine is attackable by de-amination, whereby the [5-methyl]-cytosine is replaced by a thymine residue (a guanine by an adenine residue on the other strand; *i.e.*, a G-to-A transition) (Coulondre *et al.*, 1978; Lindahl and Nyberg, 1974). The resulting T:G mismatches cause minor distortions of the DNA helix (Brown *et al.*, 1985) and it is more difficult for repair enzymes to recognize them. T:G mismatches are by a factor of 6000 less efficiently repaired than U:G mismatches formed by deamination of cytosine (Schmutte *et al.*, 1995).

What is remarkable about the *p53* gene is that 5 out of 6 *p53* mutation hot-spot codons contain CpG dinucleotides (175, 245, 248, 273 and 282). This implies methylation-driven deamination of 5-methyl cytosin as a major source of G-to-A-transition mutations at CpG dinucleotides (Tornaletti and Pfeifer, 1995). The CpG site at codon 245 is well characterized as a mutational hot spot in carcinomas, with a total of 144 mutational cases out of 4496 entries (3.2%) in the *p53* mutation databank (Hollstein *et al.*, 1996). Of these, 67 cases concern the G-to-A transition. In sarcomas, no mutational hot spot has been described (Greenblatt *et al.*, 1994). For codon 245, 5 mutation cases out of 162 mutation entries for sarcomas are compiled (Hollstein *et al.*, 1996); if we add the 3 described here, the total recorded cases number 8 (*i.e.*, 5% of known sarcoma mutations). Of these, 6 cases have a G-to-A transition. Summarizing results, codon 245 appears as a mutational hot spot for sarcomas.

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Set     Items   Description
S1    2927712   CANCER OR TUMOR OR MALIGNAN?
S2     135446   P53
S3     101820   S1 AND S2
S4       2203   MDM2(5N)EXPRESS?
S5       1672   S3 AND S4
S6        187   S5 AND PY<=1996
S7       1129   (AMPLICATION OR OVEREXPRESS?)(5N)MDM2
S8        114   S6 NOT S7
S9         66   RD (unique items)
? s increas?
Sending Break...
?s increas? (5n)mdm2
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            7904  MDM2
     S10     623  INCREAS? (5N)MDM2
? s s9 not s10
              66  S9
             623  S10
     S11      60  S9 NOT S10
? t s11/3,k,ab/1-20
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11/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11718537 PMID: 9275659
[Studies on MDM2 **oncogene** expression **and its effect on pancreatic carcinoma cells]**
Guo H; Liu T; Gao J
Department of Pathology, PUMC Hospital, CAMS, Beijing.
Zhonghua bing li xue za zhi Chinese journal of pathology (CHINA) Aug **1996** , 25 (4) p232-5, ISSN 0529-5807 Journal Code: 0005331
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: CHINESE

Over- expression **of the** MDM2 **gene is found in some cases of haematological** malignancies .

Quesnel B; Preudhomme C; Oscier D; Lepelley P; Collyn-d'Hooghe M; Facon T ; Zandecki M; Fenaux P
Inserm U124, Institut de Recherches sur le Cancer de Lille, France.

British journal of haematology (ENGLAND) Oct **1994** , 88 (2) p415-8,
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Record type: MEDLINE; Completed

We looked for **MDM2** gene amplification and over- **expression** by Southern and Northern blot analysis in 135 and 66 cases of haematological **malignancies** , including ALL, AML, CML in chronic phase, CLL, MDS, PLL, non-Hodgkin's lymphoma (NHL) and myeloma. No amplification of the gene was found. An over- **expression** of **MDM2** RNA was seen in 9/66 (14%) patients tested, including 3/9 ALL, 3/24 AML, 2/4 myelomas, 1/1 PLL, but 0/2 CML, 0/2 NHL and 0/21 MDS. None of the patients over- **expressing MDM2** had modifications of **P53** gene transcript or **p53** mutations. Most of the patients over- **expressing** **MDM2** gene had poor prognostic features (including 'unfavourable' cytogenetic abnormalities), poor response to chemotherapy and short survival. Our findings suggest that over- **expression** of **MDM2** is seen in a relatively small number of haematological **malignancies** , and is associated with poor prognosis.

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Oct **1994** ,
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Amplification of the MDM2 gene in human breast cancer **and its**
association with MDM2 and p53 **protein status.**

McCann A H; Kirley A; Carney D N; Corbally N; Magee H M; Keating G;
Dervan P A
Biotechnology Centre, University College Dublin, Belfield, Ireland.

British journal of cancer (SCOTLAND) May **1995** , 71 (5) p981-5,
ISSN 0007-0920 Journal Code: 0370635
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Document type: Journal Article
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The present study reports on the frequency of **MDM2** gene amplification and **MDM2** protein **expression** in a series of 100 breast carcinomas and its association with accumulation of the **p53** protein. Of the 100 cases, frozen samples for 82 cases were available for Southern blotting. Three of the 82 (4%) demonstrated MDM2 gene amplification of up to 6-fold. Immunohistochemical analysis of the formalin-fixed, paraffin-embedded tumours demonstrated that 7/97 (7%) had nuclear **expression** for **MDM2** in 10-50% of the tumour cells (type 2 staining) and were denoted MDM2+. Two of the MDM2-amplified samples were MDM2+ with one of the two tumours also displaying type 2 **p53** nuclear staining. Finally at the protein level, MDM2+ tumours were significantly associated with tumours having low levels of **p53** staining (0-10% cells positive) (P = 0.03). We conclude that MDM2 gene amplification occurs at a lower frequency in breast **cancer** than in non-epithelial tumours. Alterations in MDM2 and **p53** may represent alternative pathways in tumorigenesis, but they are not mutually exclusive in all cases.

Frequent occurrence of p53 **mutations in rhabdomyosarcoma and leiomyosarcoma, but not in fibrosarcoma and** malignant **neural tumors.**

Wurl P; Taubert H; Bache M; Kroll J; Meye A; Berger D; Siermann A; Holzhausen H J; Hinze R; Schmidt H; Rath F W

Surgical Clinic, Martin Luther University of Halle-Wittenberg, Halle/S., Germany.

International journal of cancer. Journal international du cancer (UNITED STATES) Aug 22 **1996** , 69 (4) p317-23, ISSN 0020-7136 Journal Code: 0042124

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Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

We have analyzed soft-tissue sarcomas (STS) molecularly for mutations in the **tumor** -suppressor gene **p53** and immunohisto-chemically for **expression** of **p53** and **mdm2** proteins. In this study, **tumor** samples from 3 groups of soft-tissue sarcomas, i.e., fibrosarcomas, myogenic sarcomas and **malignant** neural tumors (MNT), were investigated. The methods applied encompass immunohistochemistry on 198 **tumor** samples using **p53** antibodies (DO-1 and DO-7) and an mdm2 antibody (IF-2). Out of these, 100 samples were subjected to non-radioactive PCR-SSCP-sequencing analysis. Immunohistochemical detection rate for **p53** (range of 57% to 67%) and for mdm2 proteins (range of 19 to 44%) was similar in all 3 groups. In higher **tumor** grades, an increased rate of immunopositivity was found for **p53** but not for mdm2. Investigation of **p53** mutational status revealed 6 mutations in myogenic sarcomas but none in **malignant** neural tumors or fibrosarcomas, suggesting different roles of **p53** in the 3 STS groups. Interestingly, a G-->A transition in codon 245 (a CpG site) was found in 3

myogenic sarcomas. Our results and those of others suggest **p53** codon 245 as a mutational hotspot in sarcomas, as recognized in carcinomas.

Frequent occurrence of p53 **mutations in rhabdomyosarcoma and leiomyosarcoma, but not in fibrosarcoma and** malignant **neural tumors.**

Aug 22 **1996** ,

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S9          66   RD (unique items)
? s increas?
Sending Break...
?s increas? (5n)mdm2
         5899392  INCREAS?
            7904  MDM2
     S10     623  INCREAS? (5N)MDM2
? s s9 not s10
              66  S9
             623  S10
     S11      60  S9 NOT S10
? t s11/3,k,ab/1-20
```

APOPTOSIS, CANCER AND THE P53 TUMOR-SUPPRESSOR GENE (Abstract Available)

Author(s): LEE JM; BERNSTEIN A
Corporate Source: MT SINAI HOSP,SAMUEL LUNENFELD RES INST,DIV MOLEC & DEV BIOL,600 UNIV AVE/TORONTO/ON M5G 1X5/CANADA/; MT SINAI HOSP,SAMUEL LUNENFELD RES INST,DIV MOLEC & DEV BIOL/TORONTO/ON M5G 1X5/CANADA/; UNIV TORONTO,DEPT MOLEC & MED GENET/TORONTO/ON M5S 1A8/CANADA/

Journal: CANCER AND METASTASIS REVIEWS, **1995** , V14, N2 (JUN), P149-161
ISSN: 0167-7659
Language: ENGLISH   Document Type: REVIEW

Abstract: One of the most commonly detected abnormalities in human cancer is mutation of the p53 tumour suppressor gene. Intrinsic to the function of p53 is its ability to induce apoptotic cell death and to cause **cell cycle arrest** . Moreover, p53 plays an important role in controlling the cellular response to DNA damaging agents such as ionizing radiation and cancer chemotherapeutic drugs. Loss of p53 function causes increased resistance to radiation and chemotherapeutic agents, and there is increasing evidence that p53 mutational status is an important determinant of clinical outcome in cancer. This review will focus on recent data describing the biochemistry of p53 function, its role in mediating apoptosis and **cell cycle arrest** and in the control of tumour growth and death.

, **1995**

MARKER GENES FOR CYTOTOXIC EXPOSURE - P53 (Abstract Available)
Author(s): MONTENARH M
Corporate Source: UNIV SAARLAND,BLDG 44/D-66424 HOMBURG//GERMANY/
Journal: STEM CELLS, **1995** , V13, S1 (MAY), P136-141
ISSN: 1066-5099
Language: ENGLISH Document Type: ARTICLE
Abstract: The growth suppressor p53 plays an important role in the regulation of cell proliferation, DNA repair and apoptosis. In wild-type p53 expressing cells, gamma-irradiation induces an increase in the level of p53 protein and these cells exhibit a GI growth arrest. The p53-induced G(1) growth arrest is abrogated in cells expressing mutant p53, or in cells where p53 is inactivated by complex formation with cellular or viral proteins such as mdm2 or the E6 proteins of human papillomavirus (HPV) 16 or HPV18. Wild-type p53 expressing cells are radiosensitive whereas mutant p53 expressing cells are radioresistant. In some cell types, p53 mutations are observed after gamma-irradiation of cells although this observation is not consistent for all cell types. Furthermore, it is not clear whether these mutations are the direct result of irradiation or secondary effects.
, **1995**

```
? s p53
      S1  135504  P53
? s activat? (5n) p53
         2455436  ACTIVAT?
          135504  P53
      S2   11161  ACTIVAT? (5N) P53
? s cell(w)cycle(w)arrest
         5633738  CELL
          750341  CYCLE
          142979  ARREST
      S3   21150  CELL(W)CYCLE(W)ARREST
? s s2 and s3
           11161  S2
           21150  S3
      S4    1578  S2 AND S3
? s transcription?(5n)factor
          861751  TRANSCRIPTION?
         2373148  FACTOR
      S5  216100  TRANSCRIPTION?(5N)FACTOR
? s s4 and s5
            1578  S4
          216100  S5
      S6     333  S4 AND S5
? s s6 and py<1997
Processing
             333  S6
        31263329  PY<1997
      S7      77  S6 AND PY<1997
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records  (50)
...completed examining records
      S8      72  RD (unique items)
? t s8/3,k,ab/60-72
```

8/3,K,AB/60 (Item 53 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04235094 Genuine Article#: RQ469 Number of References: 63

Title: P53 DEPENDENT GROWTH SUP

```
? s cancer? or tumor or malignan?
        1585336  CANCER?
        1597891  TUMOR
         589815  MALIGNAN?
     S1 2963795  CANCER? OR TUMOR OR MALIGNAN?
? s p53
     S2  135446  P53
? s s1 and s2
        2963795  S1
         135446  S2
     S3  102760  S1 AND S2
? s mdm2
     S4    7904  MDM2
? s s3 and s4
         102760  S3
           7904  S4
     S5    5534  S3 AND S4
? s not(w)overexpress?
>>>Operator "NOT" in invalid position
? s overexpress?
     S6  209394  OVEREXPRESS?
? s s5 and s6
           5534  S5
         209394  S6
     S7    1726  S5 AND S6
? s s5 not s6
           5534  S5
         209394  S6
     S8    3808  S5 NOT S6
? s mdm2(5n) express?
           7904  MDM2
        3414915  EXPRESS?
     S9    2203  MDM2(5N) EXPRESS?
? s s3 and s9
         102760  S3
           2203  S9
    S10    1685  S3 AND S9
? s s10 and py<=1997
Processing
Sending Break...
?s s10 and py<1997
Processing
           1685  S10
       31263327  PY<1997
    S11     188  S10 AND PY<1997
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records  (50)
```

```
...examined 50 records  (100)
...examined 50 records  (150)
...completed examining records
     S12     106  RD (unique items)
? s excess
     S13  328150  EXCESS
? s s12 not s13
             106  S12
          328150  S13
     S14     105  S12 NOT S13
? s sarcoma
     S15  147075  SARCOMA
? s s14 not s15
             105  S14
          147075  S15
     S16     101  S14 NOT S15
? t s16/3,k,ab/1-5
```

16/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11736055 PMID: 9815889
Differential expression **of multiple** MDM2 **messenger RNAs and proteins in normal and tumorigenic breast epithelial cells.**
Gudas J M; Nguyen H; Klein R C; Katayose D; Seth P; Cowan K H
Medicine Branch, Division of Cancer Treatment, Medical Breast Cancer Section, National Cancer Institute, Bethesda, Maryland 20892, USA.
Clinical cancer research - an official journal of the American Association for Cancer Research (UNITED STATES) Jan **1995**, 1 (1) p71-80, ISSN 1078-0432 Journal Code: 9502500
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
The MDM2 gene is a nuclear phosphoprotein that is regulated by **p53** and functions, in one capacity, to inhibit the transcriptional activity of the wild-type **p53** protein. Multiple MDM2 transcripts were detected in human breast epithelial cells. In estrogen receptor-negative normal, immortal, and tumorigenic breast epithelial cells, we found a good

correlation between **MDM2** mRNA levels and **expression** of wild-type **p53** . When wild-type **p53** was overexpressed in estrogen receptor-negative **tumor** cells containing a mutant or no endogenous **p53** , MDM2 mRNA levels increased significantly, indicating that wild-type **p53** positively influences MDM2 mRNA levels in these **tumor** cells. Because all estrogen receptor-positive breast **tumor** cells had high MDM2 mRNA levels regardless of the status of their endogenous **p53** protein, other factors likely influence **MDM2** **expression**

[Studies on MDM2 **oncogene** expression **and its effect on pancreatic carcinoma cells]**

Guo H; Liu T; Gao J
Department of Pathology, PUMC Hospital, CAMS, Beijing.

Zhonghua bing li xue za zhi Chinese journal of pathology (CHINA) Aug **1996** , 25 (4) p232-5, ISSN 0529-5807 Journal Code: 0005331
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: CHINESE
Main Citation Owner: NLM
Record type: MEDLINE; Completed

In order to study the interrelation and interaction between MDM2 oncogene and wild type **p53** in human pancreatic **cancer** , we studied the **expression** and amplification of **MDM2** oncogene and its antagonistic effect on wild type **p53** by use of gene recombination, gene transduction and molecular hybridization techniques. The results showed that MDM2 oncogene could be detected in all 5 pancreatic cell lines, but **MDM2** mRNA **expression** varied in the different cell lines. The recombinant vector pCMV-MDM2 was transduced into PC-2/s-wtp53 cell line (a transformed PC-2 pancreatic carcinoma cell line containing wild type **p53** gene). The resultant cell line, PC-2/s-wtp53/pCMV-MDM2 showed rapid cell growth, a rate similar to that of the parent cell line PC-2. Our results verify the fact that MDM2 gene can abrogate the cell growth arrest mediated by wild type **p53** and the antagonistic function of wild type **p53** .